On Some Stages in the Life-History of Leptomonas muscæ domesticæ, with some remarks on the Relationships of the Flagellate Parasites of Insects.

By

J. S. Dunkerly.

With Plate 31.

In an endeavour to examine the biflagellate character of Herpetomonas as described by Prowazek (20), I have dissected and examined a number of house-flies. At first Musca domestica was investigated, as I had supposed that this was the animal indicated by the word "Stubenfliege." In this country, however, M. domestica does not seem to be commonly infected, as I was unable to find the parasite in it, and Hewitt (6), who examined a good number of these flies, was similarly unsuccessful. In the smaller house-fly, Homalomyia canicularis, flagellate parasites were found to be present, but Hewitt had confined himself to M. domestica. Still, the infections were very rare. I examined these flies taken in three distinct localities: Chelsea and Wandsworth in London, and Benfleet in Essex. Parasites were found in flies from each place, but always in a low percentage, about 4 per cent., of the flies examined. Other species of flies[1] have been examined, but not in large numbers, so that it is not surprising that no Herpetomonads have been met with in them as yet.

[1] I take this opportunity of expressing my thanks to Mr. Austen, who kindly assisted me in the identification of these flies.

I will first describe the forms met with by me in Homalomyia canicularis, and afterwards discuss their significance. (1) In the midgut (ventriculus) of two flies were seen some large typical Herpetomonas forms (Pl. 31, fig. 1). This form had a body 25 μ to 30 μ long, and the flagellum was 30 μ long. Its movements were characteristic, the body being clumsily swung from side to side by the lashing of the long thick flagellum. With Giemsa's stain the double character of the flagellum described by Prowazek is evident in most cases. If, as Patton (17) states, this is merely a stage in division, then it is an unusual type of division, since the kinetonucleus is not even transversely elongated when the flagellum has divided along its whole length, this being very unlike the state of affairs found by me in dividing forms (Pl. 31, fig. 9). My material for the study of these forms has been very scanty, and I can only say that they are very different, both in appearance and size, from the other flagellates met with in the fly.

(2) A commoner form (Pl. 31, figs. 2–14) was found in the intestine, and once in the Malpighian tubules. These infections were always heavy ones, the parasites occurring in dense clusters, either on the intestinal wall or free in the lumen of the intestine. On slides the clusters were seen to be formed by the typical rosettes, or more correctly, agglomerations, with the flagella pointing to the centre, as described by Woodcock (26) for cultural forms of trypanosomes. The body of this form was 15 μ to 18 μ long, and its movement was rapid and graceful, the anterior part of the body often undulating. A large number of dividing forms were usually present (Pl. 31, figs. 6–10). In one case, in which the forms were particularly elongated (Pl. 31, fig. 14), cysts were also found, and doubtful intermediate stumpy forms. The characters to which I wish to draw particular attention are the varying position of the kinetonucleus and the presence of an undulating membrane (Pl. 31, figs. 3, 4, and 11–14). (The forms shown in figs. 3, 4, and 11 are from the same fly). All intermediate stages between the short form, with its

anterior end truncated, and the elongated one with anterior extremity drawn out into a membrane attached to the flagellum, are found (Pl. 31, figs. 3, 4, and 11). A form resembling that in fig. 11 was found by Chatton and Alilaire (2) in Drosophila confusa, and described under the name Trypanosoma drosophilæ, although the authors recognised the possibility of its being a stage in the life-history of a Leptomonas found by them in the same fly. Werner (24) also described the same form from "Stubenfliegen," and named it Crithidia muscæ domesticæ to distinguish it from the biflagellate Herpetomonas muscæ domesticæ of Prowazek. Miss Mackinnon (14) also, whose paper appeared while this work was in progress, in describing what she regards as a Herpetomonas from Homalomyia corvina (?) pointed out the similarity between some of the forms found by her and the Crithidia of Werner, which would not be surprising if both are stages in the life-history of organisms belonging to the same genus, possibly to the same species. The forms shown in Pl. 31, figs. 12, 13, and 14 possess, undoubtedly, an undulating membrane, though the flagellum is not produced beyond it, and these resemble in a striking manner some stages of Trypanosoma cazalboui in cultures, described by Roubaud (22, pl. viii, figs. 2 and 6), thus indicating a close relationship between the parasite of a non-blood-sucking fly like Homalomyia and the trypanosomes of vertebrates. Patton (18, and 17, p. 142, note), in objecting to Prowazek's account of Herpetomonas (which, however, has been supported by other observers, Lingard and Jennings [12], Roubaud [22]) decided that all uni-flagellate parasites of insects with the kinetonucleus anterior to the trophonucleus and without undulating membrane are to be called Herpetomonas, and that those having the kinetonucleus posterior to the trophonucleus, and possessing an undulating membrane, should receive the generic name of Crithidia. Lühe (13) and Hartmann and Jollos (5) have pointed out that Patton's failure to see the characters observed by Prowazek and others does not prove their non-existence; and as to his

use of the name Crithidia, this is certainly a misuse of Leger's term, which he applied (8) to a short rounded form, "en form de grain d'orge légèrement aplati et tronqué a l'extrémité antérieure . . . ," and usually without an undulating membrane. However, it seems from the evidence of the forms found in Homalomyia that the same organism may be without an undulating membrane at one stage of its life-history, while possessing one at another stage. I shall return later in this paper to this question of nomenclature.

(3) In the rectum, near the rectal glands, were found masses of small oval bodies (Pl. 31, fig. 15) attached to the rectal epithelium. On examining these in water I was able to observe the mass apparently swell, as though the walls of the oval bodies were gelatinous, and after a short time some of the bodies were seen to become actively motile, with a small anterior flagellum (Pl. 31, figs. 16 and 17). The flies containing these cysts had no other flagellate stages in them, but came from the same locality as those that had. Similar cysts have been described by Minchin (15) for T. grayi, by Prowazek (20), Rosenbusch (21), and Mackinnon (14) for Herpetomonas, the latter having observed them giving rise to flagellates. The cysts stained with Giemsa (Pl. 31, fig. 15) show a faint trophonucleus and a distinct kinetonucleus, with a large number of scattered granules stained a deep purple colour, and have a definite wall surrounded by a remarkable substance which stains deeply, and may be gelatinous in nature (vide supra). But iron-hæmatoxylin shows little of these peculiar effects (Pl. 31, fig. 17A). The commencement of development of the flagellum is indicated by a clear area in Giemsa preparations (Pl. 31, fig. 15B), the borders of which appear to stain with iron-hæmatoxylin, showing a triangular area with the kinetonucleus as base (Pl. 31, fig. 17A and D), and the same appearance has been seen in non-flagellate forms of T. lewisi by Prof. Minchin, who kindly showed me his original drawings.

In the life-cycle of Leptomonas, as far as I have investigated it, we find the following forms: (1) A typical Lepto-

monas (fig. 2), which actively divides in the intestine or in the Malpighian tubules of the fly (figs. 6–10), producing (2) very active, slender forms, often with undulating membrane (figs. 11–14). These probably encyst while attached in large numbers to the rectal wall, and the cysts (figs. 15 and 17A) may be passed out with the fæces to give rise to flagellate forms in another fly, as described by Patton (**19**) for the Herpetomonas (? Leptomonas) of Musca nebulo, the Madras bazaar fly. But whether the large Herpetomonas form (fig. 1) should have a place in this life-history I am at present unable to decide. Almost certainly the above is but a part of the whole life-cycle, and the low percentage of infections have prevented the completion of it up to the present. It might be thought improbable on à priori grounds that flies in England and in India should be infected by the same pair of parasites, yet in smears of house-flies' guts which Dr. Row brought from India and kindly left at the Lister Institute, there are large Herpetomonads and small Leptomonads just as in H. canicularis in England. If these should prove to be different forms of the same organism, and at the same time have a trypanosome-stage in their life-history, considerable changes in our nomenclature of flagellate parasites will be necessitated.

As to Prowazek's description of elaborate autogamy and hereditary infection in Herpetomonas, one is tempted to interpret some of his figures (which hardly bear out his account), as being those of a Sporozoan infection, and I hope to publish shortly an account of a Microsporidian which I have found in Homalomyia.

The nomenclature of these forms, interesting on account of their probable relationship with the trypanosomes, is in a very confused state, and it is with a view to the clearing up of at least one part of the vexed question that I wish to re-state the following facts in their history.

Saville Kent in 1881 (**23**) established the genera Leptomonas and Herpetomonas for uniflagellate parasites found in a Nematode, Trilobus, and in Musca domestica respectively. The only points of distinction mentioned by him

which are of any service are that Leptomonas was $\frac{1}{2300}$ in. long, and formed rosettes, while Herpetomonas was $\frac{1}{630}$ to $\frac{1}{430}$ in. long, and had, at any rate, not been seen in rosettes or agglomerations. In 1902 Leger (**9**) found flagellate parasites in Homalomyia and other Diptera, and named an elongated form Herpetomonas (sp. var.), while a short rounded form, "en form de grain d'orge," he called Crithidia (sp. var.). Later (**10**, A and B), he described H. subulata from Tabanus as possessing an undulating membrane, still retaining the name Crithidia for short pyriform forms. Prowazek (**20**) in 1904 had investigated the parasite of the house-fly, and described it as possessing two flagella united by a membrane and arising from an anterior double basal-granule or diplosome. Novy, MacNeal, and Torrey, in 1907 (**16**) followed Leger's nomenclature for types found in mosquitoes, their Herpetomonas in cultures showing an undulating membrane. They described a diplosome, not where Prowazek had placed it, but at the posterior end of the body, and bearing, as they themselves point out, a considerable resemblance to a Diplococcus, which was generally adherent to the body of Herpetomonas in the cultures. Lingard and Jennings (**12**) in 1906 found in a Muscid fly forms showing the typical diplosome described by Prowazek, but most of their figures are not clear, and they claim to have seen the actual folding of the flagellate to form the biflagellate condition according to the Prowazek-Schaudinn theory respecting the origin of the double flagellum.

The history of Herpetomonas up to this point has been related in greater detail by Woodcock (**25**). His conclusions are—(1) That some of these parasites of mosquitoes are probably connected with Trypanosomes of vertebrates; (2) some of the typical Herpetomonads found may be simply and primarily parasites of the insects; (3) that forms adapted for life in sanguivorous insects, by which are meant "Crithidia" forms with an undulating membrane, following Patton's nomenclature, may be unrelated to any trypanosome

of a vertebrate. But no forms were then known with an undulating membrane in a truly non-sanguivorous insect. In 1908, however, Chatton and Alilaire (2) described flagellates found in Drosophila confusa—a Leptomonas (as distinct from Prowazek's Herpetomonas) and a Trypanosoma without a clear undulating membrane, but with the blepharoplast at the posterior end of the body. They named these forms L. drosophilæ and T. drosophilæ, but at the same time put forward the suggestion that they are really two stages of the same life-cycle. Werner (24) in 1909, and Rosenbusch (21) in 1910, have stated that there are two distinct parasites of the house-fly, a Herpetomonas of Prowazek and a Crithidia with posterior kinetonuclens, of which Rosenbusch describes the encystation. Roubaud, in an interesting article in 1909 (22), has used an old generic term, "Leptomonas," for the uniflagellate parasite of the fly Pycnogonum, excluding Herpetomonas of Prowazek, which he also found in the same fly. He regards, then, Herpetomonas of Prowazek as biflagellate, and Leptomonas as uniflagellate, with kinetonuclens usually anterior, but with a so-called trypanosome stage in its life-history. The evidence of Rosenbusch (21), Chatton and Alilaire (2), and Mackinnon (14), and that given by my figures, all goes to show that a form resembling Leptomonas of Saville Kent is found in non-sanguivorous flies (in three cases, house-flies), developing in the course of its life-history a form resembling a cultural trypanosome, and having an encysted stage. The fact that many observers have seen a large form (shown in Pl. 31, fig. 1), which differs very much in appearance from Leptomonas, renders it possible that the other observers who fail to see the two flagella are dealing with a different organism.

This much, however, seems certain: (1) That Leger's original pear-shaped Crithidia is only a stage of the Leptomonas life-history; also (2) that the "Crithidia" of later authors—Patton (18), Woodcock (25)—found in blood-sucking flies, or in cultures, are in some cases developmental stages of a Trypanosoma. The evidence of the forms found by me

(Pl. 31, figs. 11–14) in the house-fly, Homalomyia canicularis, shows that Rosenbusch's Crithidia muscæ domesticæ, and therefore probably Trypanosoma drosophilæ of Chatton and Alilaire, are merely forms assumed by a Leptomonas.

Should Leptomonas or Herpetomonas be the name given to these parasites of the Insecta? The Leptomonas of Saville Kent was described as being of a size comparable with that of the small Leptomonas, of, e.g., Homalomyia, whereas Herpetomonas was evidently a huge form. Again, Leptomonas was said to form rosettes. A diagnosis based on morphological grounds is of more value than one depending upon habitat. At present, therefore, Leptomonas would appear to be a correct name for the uniflagellate parasites found in the gut of non-sanguivorous insects, including house-flies, Pycnogonum (**22**), Bombyx (**11**), and in some plants (**7**), while Herpetomonas may be retained as a provisional name for a large form with peculiar flagellar apparatus and a complicated life-history, as described by Prowazek. Should the latter prove to be but a stage in the Leptomonas' life-history, then Herpetomonas should be merged in Leptomonas, since the latter would then have been the first which was accurately described. Crithidia cannot be applied as a generic name to any form, as it has simply been the name given to two stages in the life-history of Leptomonas, or in other cases to what are probably stages of Trypanosoma. That Leptomonas had priority over Crithidia was pointed out by Hartmann and Jollos (**5**), but it was not clear then that "Crithidia" was a form in the Leptomonas' life-history.

A paper by Flu on parasites of the house-fly, Musca domestica, appeared ('Centralblatt f. Bakt., etc.,' Bd. lvii, 1911, p. 522) after this paper had been sent to press, and is in the main confirmatory of the chief points emphasised above.

Protozoological Laboratory,
Lister Institute,
London.

References.

1. Chagas. — "Nova tripanozomiaze humana," 'Mem. do Inst. Oswaldo Cruz,' t. 2, f. 2, p. 159 (Abs. in 'Bull. Inst. Past.,' viii, p. 373).
2. Chatton et Alilaire.—"Coexistence d'un Leptomonas (Herpetomonas) et d'un Trypanosoma chez un Muscide non vulnerant, Drosophila confusa, Staeger," 'C. R. Soc. de Biol.,' t. 64, 1908, p. 1004.
3. Doflein.—'Lehrb. d. Prot.,' 2 Aufl., 1909.
4. Donovan.—'Kala Azar in Madras' (read before Bombay Med. Congr., February 24th, 1909.)
5. Hartmann and Jollos.—"Die Flagellatenordnung Binucleata," 'Arch. f. Prot.,' Bd. xix, 1910, Heft. 1.
6. Hewitt.—"The Bionomics, Allies, Parasites, and the Relations of M. domestica to Human Disease," 'Quart. Journ. Micr. Sci.,' 54, 1909, p. 347.
7. Lafont.—"Sur la presence d'un Leptomonas dans trois Euphorbiacées," 'Ann. de l'Inst. Past.,' xxiv, 1910, p. 205.
8. Léger.—"Sur un flagellé parasite de l'Anopheles maculipennis," 'C. R. Soc. de Biol.,' liv, 1902, p. 354.
9. ———"Sur quelques Cercomonadines nouvelles ou peu connues parasites de l'intestine des Insectes," 'Arch. f. Prot.,' Bd. ii, 1902, p. 180.

10A. ——— "Sur un nouveau Flagellé parasite des Tabanides," 'C. R. Soc. de Biol.,' t. 57, p. 613.

10B. ——— "Sur les affinités de l'Herpetomonas subulata, et la phylogenie des Trypanosomes," 'C R. Soc. de Biol,' t. 57, p. 615.

11. Levaditi.—"Sur un nouveau Flagellé parasite du Bombyx mori," 'C. R. Acad. des Sciences,' cxli, 1905, p. 631.
12. Lingard and Jennings.—'Some Flagellate Forms found in Diptera,' Adlard and Son, London, 1906.
13. Lühe.—"Die im Blute schmarotzenden Protozoen und ihre nächsten Verwandten," Mense's 'Handbuch der Tropenkrakh., 1906.
14. Mackinnon.—"Herpetomonads from the Alimentary Tract of certain Dung-flies," 'Parasitology,' iii, September, 1910, p. 255.
15. Minchin.—"Investigations on the Development of Trypanosomes in Tsetse-flies, etc.," 'Quart. Journ. Micr. Sci.,' 52, p. 159.

16. Novy, MacNeal and Torrey.—"The Trypanosomes of Mosquitoes and other Insects," 'Journ. Infect. Diseases,' iv, No. 2, p. 223.

17. Patton.—"The Life cycle of a Species of Crithidia parasitic in Gerris fossarum," 'Arch. f. Prot.,' xii, 1908, p. 131.

18. ——— "A Critical Review of our Present Knowledge of the Hæmoflagellates and Allied Forms," 'Parasitology,' ii, May, 1909, p. 91.

19. ——— "Experimental Infection of the Madras Bazaar fly, Musca nebulo, with Herpetomonas muscæ domesticæ," 'Bull. Soc. Patholog. Exot.,' iii, p. 264.

20. Prowazek.—"Die Entwickelung von Herpetomonas," 'Arb. a. d. Kais. Ges.,' xx, 1904, p. 440.

21. Rosenbusch.—"Eine neue Encystierung bei Crithidia muscæ domesticæ," 'Centr. f. Bakt.,' Bd. lv, 1910, p. 387.

22. Roubaud.—"Les Trypanosomes pathogenes et la Glossina palpalis," 'La Maladie du Sommeil au Congo Français,' 1909.

23. Saville Kent.—'Manual of the Infusoria,' 1880–81.

24. Werner.—"Über eine eingeisselige Flagellatenform im Darm der Stubenfliege," 'Arch. f. Prot.,' xiii, 1909.

25. Woodcock.—"The Hæmoflagellates and Allied Forms," in Lankester's 'Treatise on Zoology,' vol. i, fasc. i, 1909, p. 193.

26. ——— "Studies on Avian Hæmoprotozoa," 'Quart. Journ. Micr. Sci.,' 55, 1910, p. 641.

EXPLANATION OF PLATE 31,

Illustrating Mr. J. S. Dunkerly's paper "On Some Stages in the Life-history of Leptomonas muscæ domesticæ, with Some Remarks on the Relationships of the Flagellate Parasites of Insects."

[All figures are outlined with the aid of Zeiss-Abbé drawing apparatus, and are drawn at a magnification of 2400.]

Fig. 1.—Large Herpetomonas from stomach of Homalomyia canicularis. Osmic vapour, Giemsa.

Fig. 2.—Leptomonas from intestine of H. canicularis, showing distinct blepharoplast. Flemm.-Fe. hæm.

Figs. 3 and 4.—Leptomonas from intestine of H. canicularis, showing varying positions of the kinetonucleus. Schaud-Fe. hæm.

Figs. 5–10.—Leptomonas from intestine of H. canicularis; various stages in division. Flemm.-Fe. hæm.

Figs. 11–14.—Leptomonas from intestine of H. canicularis: trypaniform individuals. Schaud.-Fe. hæm.

Fig. 15.—Cysts of Leptomonas muscæ domesticæ from rectum of H. canicularis, showing scattered nuclear material. Osmic vapour. Giemsa.

Fig. 16.—Small flagellate forms a few minutes after leaving cyst. Osmic vapour. Giemsa.

Fig. 17.—Small flagellate forms a few minutes after leaving cyst showing development of the flagellum. Flemm.-Fe. hæm.